

IN THE SPECIFICATION

The following replacement paragraphs are presented:

Following the title, please insert the following paragraph:

The United States Government has rights in this invention under contract number DE-AC36-99G0-10337 between the United States Department of Energy and the National Renewable Energy Laboratory, a division of the Midwest Research Institute.

On page 3, lines 16-27:

A method for reducing the glycosylation of an expressed *Trichoderma reesei* CBHI protein by site-directed mutagenesis (“SDM”) is disclosed. The method includes replacing an N-glycosylation site amino acid residue, such as asparagines 45, 270, and/or 384 (referenced herein as CBHI-N45A, CBHI-N270A and CBHI-N384A, respectively), with a non-glycosyl accepting amino acid residue, such as is alanine. Various mutagenesis kits for SDM are available to those skilled in the art and the methods for SDM are well known. The description below discloses a procedure for making and using CBHI variants: CBHI-N45A; CBHI-N270A; and CBHI-N384A. The examples below demonstrate the expression of active CBHI in the heterologous fungus *Aspergillus awaniori*.

Variants of CBH I embodiments that include mutations that provide for improved end product inhibition and for thermal tolerance.

On page 3, lines 31-33:

Figure 1. Coding sequence for the *cbl1* gene (SEQ ID NO: 4). Small case letters represent the signal sequence, large case letters the catalytic domain, bolded italics the linker region, and large case underlined the cellulose-binding domain.

On page 4, lines 7-8:

Figure 4. Coding sequence, SEQ ID NO: 19, for the linker region of the *cbhI* gene, SEQ ID NO: 4, showing additional proline nucleotides that effect conformation of the linker region in the protein structure.

On page 4, lines 12-24:

Acquisition of the gene-by was done by either cDNA cloning or by PCR of the gene from genomic DNA. CBH I cDNA was isolated from a *T. reesei* strain RUT C-30 cDNA library constructed using a PCR-generated probe based on published CBH I gene sequences (Shoemaker, et al., 1983). The cDNA's were cloned (using the Zap Express cDNA kit from Stratagene; cat. #200403) into the XhoI and EcoRI site(s) of the supplied, pre-cut lambda arms. An XhoI site was added to the 3' end of the cDNA during cDNA synthesis, and sticky-ended REI linkers were added to both ends. After XhoI digestion, one end has an XhoI overhang, and the other (5' end) has an Eco RI overhang. The insert can be removed from this clone as an approximately 1.7 kb fragment using SalI or SpeI plus XhoI in a double digest. There are two Eco RI, 1—one Barn HI, 3 SacI and one HindIII sites in the coding sequence of the cDNA itself. The plasmid corresponding to this clone was excised *in vivo* from the original lambda clone, and corresponds to pB210-5A. Thus, the cDNA is inserted in parallel with a Lac promoter in the pBK-CMV parent vector. Strain pB210-5A grows on LB + kanamycin (50 ug/mL).

On page 15, lines 16-22:

The present example demonstrates the utility of the present invention for providing a nucleic acid molecule having a nucleic acid sequence that has a sequence 5'-GGCGGAAACCCGCCTGGCACCACC-3' (SEQ ID NO: 3). The identified nucleic acid sequence presents a novel linker region nucleic acid sequence that differs from previously reported nucleic acid sequence by the addition of one (1) codon. The invention in some

aspects thus provides a nucleic acid molecule having a nucleic acid sequence that comprises a linker region of about 20 to 60 nucleotides identified here.

On pages 16-19:

Table 2. Proline mutations to improve thermal tolerance.

Mutation	Native sequence and mutatgenic oligonucleotide
<u>SEQ ID NO: 23</u>	5'-GCACTCTCCAATCGGAGACTCACCCG-3'
<i>S8P - native sense strand</i>	
<u>SEQ ID NO: 24</u>	5'-GCACTCTCCA <u>ACC</u> GGAGACTCACCCG-3'
Mutagenic sense strand	
<u>SEQ ID NO: 25</u>	5'-CGGGTGAGTCTCC <u>G</u> TTGGAGAGTGC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 26</u>	5'-GGCACGTGCAC T CAACAGACAGGCTCCG-3'
<i>N27P - native sense strand</i>	
<u>SEQ ID NO: 27</u>	5'-GGCACGTGCAC <u>T</u> CAACAGACAGGCTCCG-3'
Mutagenic sense strand	
<u>SEQ ID NO: 28</u>	5'-CGGAGCCTGTCTGT <u>G</u> GAGTGCACGTGCC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 29</u>	5'-GGCGCTGGACTCAC <u>G</u> CCTACGAACAGCAGCACG-3'
<i>A43P - native sense strand</i>	
<u>SEQ ID NO: 30</u>	5'-GGCGCTGGACTCAC <u>CC</u> CCTACGAACAGCAGCACG-3'
Mutagenic sense strand	
<u>SEQ ID NO: 31</u>	5'-CGTGCTGCTGTTGT <u>AGGGT</u> GAGTCCAGCGCC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 32</u>	5'-GCTGTCTGGACGGTGCCGCCTACGCG-3'
<i>G75P - native sense strand</i>	
<u>SEQ ID NO: 33</u>	5'-GCTGTCTGGAC <u>CC</u> CCTGCCGCCTACGCG-3'
Mutagenic sense strand	
<u>SEQ ID NO: 34</u>	5'-CGCGTAGGCC <u>G</u> GGTCCAGACAGC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 35</u>	5'-GCCTCTCCATTGGCTTGTCACCC-3'
<i>G94P - native sense strand</i>	
<u>SEQ ID NO: 36</u>	5'-GCCTCTCCATT <u>CC</u> CTTGTCACCC-3'
Mutagenic sense strand	
<u>SEQ ID NO: 37</u>	5'-GGGTGACAA <u>AGG</u> GAATGGAGAGGC-3'
Mutagenic anti-sense strand	

<u>SEQ ID NO: 38</u>	5'-GGCCAACGTT GAGGGCT GGGAGCC-3'
<i>E190P - native sense strand</i>	
<u>SEQ ID NO: 39</u>	5'-GGCCAACGTT CCGGGCT GGGAGCC-3'
Mutagenic sense strand	
<u>SEQ ID NO: 40</u>	5'-GGCTCCCAG CCCCGGA ACGTTGGCC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 41</u>	5'-GGCTGGGAGGCCGT CATCCAACAACGCG -3'
<i>S195P - native sense strand</i>	
<u>SEQ ID NO: 42</u>	5'-GGCTGGGAG CCCGCC ATCCAACAACGCG-3'
Mutagenic sense strand	
<u>SEQ ID NO: 43</u>	5'-CGCGTTGTTGGAT GGCGGCT CCCAGCC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 44</u>	5'-CGATACCACCAAGAAATTGACC GTTGTCACCC -3'
<i>K287P - native sense strand</i>	
<u>SEQ ID NO: 45</u>	5'-CGATACCACCAAG CCATTGACC GTTGTCACCC-3'
Mutagenic sense strand	
<u>SEQ ID NO: 46</u>	5'-GGGTGACAACGGT CAATGGCTTGGTGGTATCG -3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 47</u>	5'-CGAGACGTCGGGT GCCATCAACCGATA C-3'
<i>A299P - native sense strand</i>	
<u>SEQ ID NO: 48</u>	5'-CGAGACGTCGGGT CCCATCAACCGATA C-3'
Mutagenic sense strand	
<u>SEQ ID NO: 49</u>	5'-GTATCGGTTGAT GGGACCCGACGTCTCG -3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 50</u>	5'-GGCGTCACTT CCAGCAGCCAACGCCGAGCTTGG -3'
<i>Q312P/N315P - native sense strand</i>	
<u>SEQ ID NO: 51</u>	5'-GGCGTCACTT CCCAGCCCCCGCCGAGCTTGG -3'
Mutagenic sense strand	
<u>SEQ ID NO: 52</u>	5'-CCAAGCTCGGCG GGGGCTGC GGAAAGTGACGCC-3'
Mutagenic anti-sense strand	
<u>SEQ ID NO: 53</u>	5'-GGCTACCT CTGGCGGCATGGTTCTGG -3'
<i>G359P - native sense strand</i>	
<u>SEQ ID NO: 54</u>	5'-GGCTACCT CTCCGGCATGGTTCTGG -3'
Mutagenic sense strand	
<u>SEQ ID NO: 55</u>	5'-CCAGAAC CATGCCGGAGAGGTAGCC -3'
Mutagenic anti-sense strand	

<u>SEQ ID NO: 56</u>	5'-GCGGAAGCTGCTCCACCAGCTCCGGTGTCCCTGC-3'
<i>S398P/S401 P- native sense strand</i>	
<u>SEQ ID NO: 57</u>	5'-GCGGAAGCTGCC <u>CCCACCAGC</u> <u>CCGGTGTCCCTGC</u> -3'
<u>m</u> <u>Mutagenic sense strand</u>	
<u>SEQ ID NO: 58</u>	5'-GCAGGGACACC <u>GGG</u> GCTGGT <u>GGG</u> CAGCTTCCGC-3'
<u>m</u> <u>Mutagenic anti-sense strand</u>	
<u>SEQ ID NO: 59</u>	5'-GTCTCCCAC <u>GCC</u> AAGGT <u>CACC</u> -3'
<i>A414P - native sense strand</i>	
<u>SEQ ID NO: 60</u>	5'-GTCTCCCAC <u>CCC</u> AAGGT <u>CACC</u> -3'
<u>m</u> <u>Mutagenic sense strand</u>	
<u>SEQ ID NO: 61</u>	5'-GGTGAC <u>CTTGGG</u> TGGGAGAC-3'
<u>m</u> <u>Mutagenic anti-sense strand</u>	
<u>SEQ ID NO: 62</u>	5'-GGCAGCAC <u>CCGG</u> CA <u>CCCTAG</u> CGGCGGCAACCC-3'
<i>N431P/S433P - native sense strand</i>	
<u>SEQ ID NO: 63</u>	5'-GGCAGCAC <u>CCGG</u> <u>CCCC</u> CT <u>CCCGG</u> CGGCAACCC-3'
<u>m</u> <u>Mutagenic sense strand</u>	
<u>SEQ ID NO: 64</u>	5'-GGGTTGCC <u>GGG</u> GAGGGGGGCCGGT <u>GCTGCC</u> -3'
<u>m</u> <u>Mutagenic anti-sense strand</u>	

Table 3. Mutation to remove peptide strain.

Mutation site	Native sequence and mutatgenic oligonucleotide
<u>SEQ ID NO: 65</u>	5'-GGCTTGT <u>CACCCAGT</u> T <u>CTGCG</u> CAGAAC <u>GTTGGC</u> -3'
<i>S99G- native sense strand</i>	
<u>SEQ ID NO: 66</u>	5'-GGCTTGT <u>CACCCAGG</u> <u>GTGCG</u> CAGAAC <u>GTTGGC</u> -3'
<u>m</u> <u>Mutagenic sense strand</u>	
<u>SEQ ID NO: 67</u>	5'-GCCAAC <u>GTTCTT</u> <u>CTGCG</u> <u>ACCC</u> GGT <u>GACAAAGCC</u> -3'
<u>m</u> <u>Mutagenic anti-sense strand</u>	

Table 3b. Y245G analogs to remove product inhibition.

Mutation site	Native sequence and mutatgenic oligonucleotide
<u>SEQ ID NO: 68</u>	5'-CCGATAAC <u>A</u> <u>AGAT</u> <u>ATGGCGGC</u> -3'
<i>R251A - native sense strand</i>	
<u>SEQ ID NO: 69</u>	5'-CCGATAAC <u>GC</u> <u>C</u> <u>TATGGCGGC</u> -3'
<u>m</u> <u>Mutagenic sense strand</u>	
<u>SEQ ID NO: 70</u>	5'-GCCGCCAT <u>AGGCG</u> <u>TTATCGG</u> -3'
<u>m</u> <u>Mutagenic anti-sense strand</u>	
<u>SEQ ID NO: 71</u>	5'-CCCGGTGCC <u>GTGCG</u> <u>CGGAAG</u> <u>GCTGCTCCACC</u> -3'
<i>R394A- native sense strand</i>	

<u>SEQ ID NO: 72</u>	5'-CCCGGTGCCGTGG <u>CCGGAAGCTGCTCCACC-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 73</u>	5'-GGTGGAGCAGCTCC <u>GGCCACGGCACCGGG-3'</u>
<u>mMutagenic anti-sense strand</u>	
<u>SEQ ID NO: 74</u>	5'-GCTGAGGAGGCAGAATT <u>CGCGGATCCTCTTC-3'</u>
<i>F338A- native sense strand</i>	
<u>SEQ ID NO: 75</u>	5'-GCTGAGGAGGCAGAAG <u>CCGGGATCCTCTTC-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 76</u>	5'-GAGAAAGAGGAT <u>CCGCCGGCTTCTGCCTCCTCAGC-3'</u>
<u>mMutagenic anti-sense strand</u>	
<u>SEQ ID NO: 77</u>	5'-GGAACCCATAC <u>CGCCTGGCAACACCAGC-3'</u>
<i>R267A- native sense strand</i>	
<u>SEQ ID NO: 78</u>	5'-GGAACCCATAC <u>GCCCTGGCAACACCAGC-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 79</u>	5'-GCTGGTGTGCC <u>AGGGCGTATGGTTCC-3'</u>
<u>mMutagenic anti-sense strand</u>	
<u>SEQ ID NO: 80</u>	5'-CCTACCCGACAAAC <u>GAGACCTCCTCACACCCGG-3'</u>
<i>E385A- native sense strand</i>	
<u>SEQ ID NO: 81</u>	5'-CCTACCCGACAAAC <u>GCCACCTCCTCACACCCGG-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 82</u>	5'-CCGGGTGTGGAGGAGGT <u>GGCGTTGTCGGGTAGG-3'</u>
<u>mMutagenic anti-sense strand</u>	

Table 4. N to A mutations to remove glycosylation.

Mutant	Native sequence and mutagenic oligonucleotide
<u>SEQ ID NO: 20</u>	5'-GGACTCACGCTACGA <u>ACAGCACGACACTGC-3'</u>
<i>N45A - native sense strand</i>	
<u>SEQ ID NO: 83</u>	5'-GGACTCACGCTACG <u>GGCCAGCACGACACTGC-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 84</u>	5'-GCAGTTCGTGCT <u>GGCCGTAGCGTGAGTCC-3'</u>
<u>mMutagenic anti-sense strand</u>	
<u>SEQ ID NO: 21</u>	5'-CCCATACCGC <u>CTGGCAACACCAGCTTACGGCCC-3'</u>
<i>N270A - native sense strand</i>	
<u>SEQ ID NO: 85</u>	5'-CCCATACCGC <u>CTGGCGCCACCAAGCTTACGGCCC-3'</u>
<u>mMutagenic sense strand</u>	
<u>SEQ ID NO: 86</u>	5'-GGGCCGTAGAAG <u>CTGGTGGCGCCAGGCGGTATGGG-3'</u>
<u>mMutagenic anti-sense strand</u>	

<u>SEQ ID NO: 22</u>	5'-GGACTCCACCTACCCGACAA <u>AC</u> GAGACCTCCTCACACCCG-3'
<i>N384A - native sense strand</i>	
<u>SEQ ID NO: 87</u>	5'-GGACTCCACCTACCCGAC <u>A</u> <u>CC</u> GAGACCTCCTCACACCCG-3'
<i>mMutagenic sense strand</i>	
<u>SEQ ID NO: 88</u>	5'-CGGGTGTGGAGGAGGTCT <u>CGG</u> TGTCGGTAGGTGGAGTCC-3'
<i>mMutagenic anti-sense strand</i>	

Table 5. Helix capping mutations to improve thermal tolerance.

Mutant	Native sequence and mutagenic oligonucleotide
<u>SEQ ID NO: 89</u>	5'-GCTGAGGAGGCAGAATT <u>CGG</u> CGG-3'
<i>E337R - native sense strand</i>	
<u>SEQ ID NO: 90</u>	5'-GCTGAGGAGGC <u>A</u> <u>CG</u> TCGGCGG-3'
<i>mMutagenic sense strand</i>	
<u>SEQ ID NO: 91</u>	5'-CCGCCGA <u>A</u> <u>CG</u> GTGCCTCCTCAGC-3'
<i>mMutagenic anti-sense strand</i>	
<u>SEQ ID NO: 92</u>	5'-GGCAACGAGCT <u>CAAC</u> GTGATTACTGC-3'
<i>N327D - native sense strand</i>	
<u>SEQ ID NO: 93</u>	5'-GGCAACGAG <u>CTCG</u> ACGATGATTACTGC-3'
<i>mMutagenic sense strand</i>	
<u>SEQ ID NO: 94</u>	5'-GCAGTAATCATCGT <u>CG</u> GAGCTCGTTGCC-3'
<i>mMutagenic anti-sense strand</i>	
<u>SEQ ID NO: 95</u>	5'-CCGGTGTCCCTG <u>CTCAGGTCGAATCTCAGTCTCCC</u> -3'
<i>A405D - native sense strand</i>	
<u>SEQ ID NO: 96</u>	5'-CCGGTGTCCCT <u>GAT</u> TCAGGTCGAATCTCAGTCTCCC-3'
<i>mMutagenic sense strand</i>	
<u>SEQ ID NO: 97</u>	5'-GGGAGACTGAGATT <u>CGACCTGAT</u> CAGGGACACCGG-3'
<i>mMutagenic anti-sense strand</i>	
<u>SEQ ID NO: 98</u>	5'-GCTCAGGTCGAAT <u>CTCAGTCTCCCACGCC</u> -3'
<i>Q410R - native sense strand</i>	
<u>SEQ ID NO: 99</u>	5'-GCTCAGGTCGAAT <u>CTCGCTCTCCCACGCC</u> -3'

<u>m</u> Mutagenic sense strand	
<u>SEQ ID NO: 100</u>	5'-GGCGTTGGGAGA <u>G</u> C GAGATTGACCTGAGC-3'
<u>m</u> Mutagenic anti-sense strand	
<u>SEQ ID NO: 101</u>	5'-CCCTATGTCCTGACAACGAGACCTGCGCG-3'
<i>N64D - native sense strand</i>	
<u>SEQ ID NO: 102</u>	5'-CCCTATGTCCTGAC <u>G</u> ACGAGACCTGCGCG-3'
<u>m</u> Mutagenic sense strand	
<u>SEQ ID NO: 103</u>	5'-CGCGCAGGTCTCGT <u>C</u> GTCAGGACATAAGGG-3'
<u>m</u> Mutagenic anti-sense strand	
<u>SEQ ID NO: 104</u>	5'-GCTCGACCCTATGTCCTGACAACGAGACCTGCGCGAAGAACTGC-
<i>N64D - native sense strand</i>	3'
<u>SEQ ID NO: 105</u>	5'-GCTCGACCCTATGTCCTGAC <u>G</u> ACGAGACCTGCGCGAAGAACTGC-
<u>m</u> Mutagenic sense strand	3'
<u>SEQ ID NO: 106</u>	5'-GCAGTTCTCGCGCAGGTCTCGT <u>C</u> GTCAGGACATAAGGGTCGAGC-
<u>m</u> Mutagenic anti-sense strand	3'

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